SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF (4-PYRIDYL)GLYOXYLIC ACID

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In our preceding communication [1], we discussed the synthesis and chemotherapeutic properties of (3-pyridyl)glyoxylic acid derivatives.

In the present work, we synthesized (4-pyridyl)glyoxylic acid (I) for the first time and carried out tests on the antitubercular activity of (3-pyridyl)glyoxylic acid (II) and certain other derivatives of I and II.

According to [2], the corresponding pyridylglyoxylic acid is obtained only for isomer III during the oxidation of 3- and 4-acetylpyridines (III) and (IV) using selenium dioxide, while in the oxidation of IV, only isonicotinic acid (V) is obtained.

Using $KMnO_4$ in an aqueous-alkaline medium, 4- and 3-ethylpyridines (VI) and (VII) can be converted into compounds I and II, respectively.

By adding in one portion, half of the calculated amount of pure VII and a double amount of 10% KOH to a 1% aqueous solution of $KMnO_4$ warmed to 45°C, and holding the mixture for 10 min at this temperature, up to 50% of the keto acid II can be obtained, based on the phenylhydrazone isolated from the reaction products, of about 20% based on the preparatively obtained keto acid II. The yield of the second oxidation product, nicotinic acid (VIII) is in this case about 40%. Compound VI under the same conditions undergoes a more extensive oxidation. The yield of V is 75%, while the keto acid I can be obtained in about 25% yield, based on the phenylhydrazone, or 5% based on the preparatively isolated keto acid I, containing about 20% (according to the PMR spectrum data) of other products.

We carried out a preparative isolation of the free acids I and II in the above cases by acidifying to pH 3.0-3.5 with hydrochloric acid partially evaporated aqueous solutions of potassium salts of the oxidation products, followed by separation of the precipitated monocarboxylic acids V or VIII, evaporation of the aqueous filtrates to dryness in vacuo at a temperature not higher than 50°C and chromatographic separation of keto acids I or II from the admixture of monocarboxylic acids V or VIII on a column with silica gel in a 4:1 $CHCl_3$–MeOH system.

The free keto acids I and II were characterized by spectral data, and in the case of compound II by a total elemental analysis as well.

When heated to 150°C, keto acid II fairly readily becomes decarboxylated. Figure 1 shows the decarboxylation isotherms of this compound in an oxygen (curve 1) and nitrogen atmosphere (curves 2, 3, 4), so that this process can be considered as a first order reaction and the rate constant can be calculated. The decarboxylation of II at 150, 160, and 170°C was accompanied by resinification of the products formed, which affected the form of curves 1-4. The decarboxylation rate constant of II at 150°C was equal to 0.0031 min^{-1}.

In contrast with the previously described [1] esterification of acid II by a HCl solution in ethanol, compound I cannot, according to chromato-spectrometric data, be converted under these conditions into the ethyl ester of (4-pyridyl)glyoxylic acid (see top of following page).

A similar decarboxylation process is also observed in the case of phenylhydrazone of acid II (IX) during its recrystallization from DMFA (~153°C), by which the (3-pyridyl)carboxaldehyde hydrazone (X) can be isolated in 86.5% yield.

Acid I readily forms derivatives at the keto group: thiosemicarbazone (XI), semicarbazone (XII), phenylhydrazone (XIII), 4-phenylthiosemicarbazone (XIV) were prepared, in a simi-

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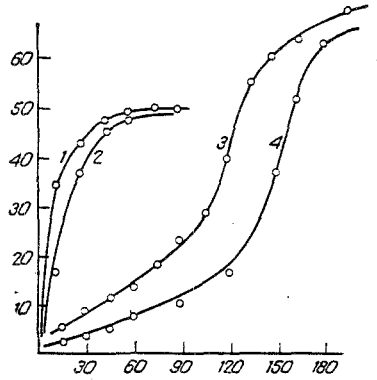
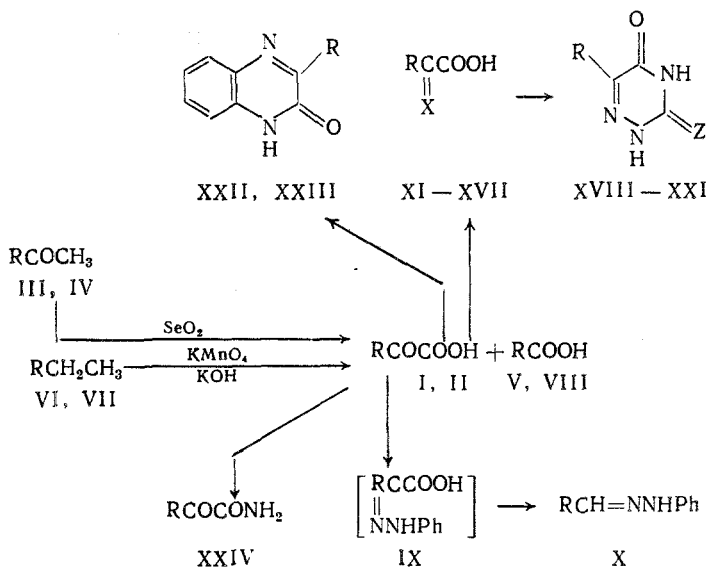


Fig. 1. Decarboxylation isotherms of (3-pyridyl)glyoxylic acid (II). Curve 1 – in O_2 current; curves 2-4 – in N_2 current; curves 1, 2 – at 170°C; 3) at 160°C, 4) at 150°C. On ordinate – CO_2, mg; on abscissa – time, min.

Table 1. Chemical Shifts of ^1H in PMR Spectra of (4-pyridyl)-glyoxylic Acid (I) and its Derivatives

Compound	Solvent	δ, ppm			
		pyridine ring		benzene ring	
		2-H, 6-H	3-H, 5-H	2′, 3′, 5′ 6′	4′
I	D_2O	8,57 (m)	7,57 (m)	—	—
XI	0,5 N. NaOD	8,52 (m)	7.69 (m)	—	—
XII	0,5 N. NaOD	8,53 (m)	7,64 (m)	—	—
XIII	1 N. NaOD	8,43 (m)	7.67 (m)	6,14—7,43 (m)	6,96 (m)
XIV	1 N NaOD	8,53 (m)	7,74 (m)	7,30—7,50 (m)	7,09 (m)
XVIII	0,5 N. NaOD, DMSO	8,54 (m) 8,66 (br.s)	7,75 (m) 7,87 (m)	—	—
XIX	0,5 N. NaOD, DMSO	8,58 (m) 8,67 (br.s)	7,79 (m) 7,9 (br.s)	—	—



R = 3-pyridyl (II, III, VII — X, XV — XVII, XX, XXI, XXIII, XXIV),
4-pyridyl (I, IV — VI, XI — XIV, XVIII, XIX, XXII);
X = $NNHCSNH_2$ (XI, XV), $NNHCONH_2$ (XII, XVI), NNHPh (XIII), NNHCSNHPh (XIV, XVII);
Z = O (XVIII, XX), S (XIX, XXI).

lar way as thiosemicarbazone (XV), semicarbazone (XVI), and 4-phenylthiosemicarbazone (XVII) were obtained from acid II [1]. In a series of experiments derivatives XI-XIV were also obtained directly from the oxidation products of VI and VII, without preliminary separation of V or VIII (see for example, in the experimental chemical section, the preparation of compound XIV and also the amide of (3-pyridyl)glyoxylic acid).

When treated with an aqueous solution of potassium carbonate, thiosemicarbazone XI and semicarbazone XII were converted in yields of 68 and 47%, respectively, into 3,5-dioxo-6-(4-

TABLE 2. Antitubercular Activity in in-Vitro Experiments of (3-Pyridyl)- and (4-Pyridyl)glyoxylic Acid Derivatives

Compound	Strains MIC, μg/ml				
	$H_{37}R_V$	Academia	Bov. 8	M. aquae	ATC C-607
Prothionamide	<0,06	—	<0,06	0,5	23,0
II	1000	1000	64	>1000	>1000
XI	153	—	153	1000	—
XIII	<0,08	<0,06	4,0	32,0	64,0
XIV	>1000	—	>1000	>1000	—
XV	1000	>1000	153	>1000	>1000
XVI	>1000	1000	>1000	>1000	>1000
XVII	—	—	>1000	>1000	—
XX	3,5	>1000	>153	>1000	>1000
XXI	153	>153	23,0	>1000	>1000

pyridyl)-2,3,4,5-tetrahydro-1,2,4-triazine (XVIII) and 5-oxo-6-(4-pyridyl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine (XIX), similarly as in the case of the previously described [1] transformations XV → XVII and XX → XXI. With o-phenylenediamine, keto acid I forms 2-oxo-3-(4-pyridyl)-1,2-dihydroquinoxaline (XXII) [1] similarly to the previously described [1] transformation of keto acid II into 2-oxo-3-(3-pyridyl)-1,2-dihydroquinoxaline (XXIII).

The structures of compounds I, XI-XIV, XVIII, XIX, XXII were confirmed by PMR spectra (Table 1).

The (4-pyridyl)glyoxylic acid derivatives XI-XIV synthesized in the present work and the previously described [1] compounds II, XV-XVII, XX, XXI were tested for antitubercular activity and the corresponding results are given in the experimental biological section and in Table 2.

EXPERIMENTAL (CHEMICAL)

The ^1H NMR spectra were run on an XL-200 spectrometer ("Varian," Switzerland) with a working frequency of 200 MHz [internal standard – dioxane (δ 3.74 ppm, see Table 1)]. The IR spectra were determined on a Perkin-Elmer-457 spectrophotometer (Sweden).* The decarboxylation features of pyridylglyoxylic acid II were studied using the quartz tube of an elemental analysis apparatus with an outlet connection for passing the carrier gas. Samples of II (0.15-0.19 g) were placed in quartz boats. The reactions were carried out in an O_2 or N_2 current, at a flow rate of the carrier gas of 15-20 ml/min and at temperatures of 150, 160, and 170°C (±5°C). The vapor-phase products of evaporation and degradation were collected in a U-shaped tube, while the liberated CO_2 was caught in a Pregl absorption apparatus, filled with askarite, which was weighed at given time intervals. The elemental analysis values corresponded to the calculated ones.

(4-Pyridyl)glyoxylic Acid (I). Compound VI (3 g, 28 mmoles) and 30 ml of 10% KOH (54 mmoles) are added in one portion to 1470 ml of 1% aqueous solution of $KMNO_4$ (9.3 mmoles) heated to 45°C. The mixture is stirred for 1.5 h at 45°C (to the disappearance of $KMnO_4$) and 10 ml of EtOH are added. After standing for 10 min at 45°C, the MnO_2 precipitate is filtered and washed with 20 ml of water. The combined filtrates are evaporated in a porcelain dish on a water bath to a volume of 50 ml, acidified with HCl to pH 3.0-3.5, and the precipitate of V that separates [2.6 g (61.9%)] is filtered. The filtrates are evaporated in vacuo to dryness at a temperature not higher than 50°C. The dry residue is dissolved in 50 ml of a $CHCl_3$–MeOH mixture (4:1) and chromatographed on a 3 × 15 cm column filled with silica gel with a particle size of 40/100 μm. The material is eluted by the same mixture of solvents, the process being monitored by TLC on Silufol plates with UV detection. After washing out the residues of V (R_f 0.38), 0.22 g (5.2%) of acid I is obtained, having in TLC one spot with R_f 0.04. Mass spectrum m/z [M – CO] = 123 and [M – CO_2] = 107.

*The IR spectra were recorded in the laboratory of physical methods of investigation of the All Union Scientific Research Chemical Pharmaceutical Institute by N. M. Rubtsov under the direction of Prof. Yu. N. Sheinker, to whom the authors wish to express their gratitude.

(3-Pyridyl)glyoxylic Acid (II) is obtained in a similar way as I, by the oxidation of 5 g (46.7 mmoles) of VII using 2450 ml of a 1% solution of $KMnO_4$ (15.5 mmoles) in the presence of 50 ml of 10% KOH (88.4 mmoles) at 45°C, proceeding according to the above described method. The yield of VIII is 2.31 g (40.4%), and of II 1.4 g (19.8%), mp 170-172°C (from water) [1]. Found, %: C 55.40, H 3.37, N 9.35. $C_7H_5NO_3$. Calculated, %: C 55.62, H 3.31, N 9.27.

(3-Pyridyl)glyoxylic Acid Amide. A 2 ml portion of concentrated H_2SO_4 and 50 ml of absolute EtOH are added, with cooling by ice water, to the solid residue obtained after the evaporation of oxidation products of 4 g of VI by the above method. The mixture is boiled for 4 h with stirring. Excess EtOH is filtered off in vacuo and the residue is treated with aqueous ammonia to pH 3.0-4.0 with external cooling with ice. An exhaustive extraction with ether is carried out, and then with $CHCl_3$, and the extracts are dried over calcined potassium carbonate. After the evaporation of the ether solution, 3.9 g (35%) of ethyl nicotinate are obtained, and after evaporation of the chloroform solution, 3.15 g (28%) of (3-pyridyl)glyoxylic acid amide, colorless crystals, mp 147-149°C (from benzene) are obtained. IR spectrum, ν_{max}, cm^{-1}: 1710 (CO), 1678 (CON), 3080, 3275 (NH_2). Found: M^+ 150. $C_7H_6O_2N_2$. Calculated: M 150.

The synthesis of compounds II, XV-XVII, XX, XXI, XXIII are described in [1].

(3-Pyridyl)carboxaldehyde Phenylhydrazone (X). A solution of 0.71 g (6.6 mmoles) of phenylhydrazine in 3 ml of 35% ethanol is added to a solution of 1 g (6.6 mmoles) of keto acid II in 2 ml of water. The mixture is allowed to stand for 2 h at 20°C, the phenylhydrazone that separates is filtered, washed with water, and recrystallized from DFMA. Yield, 1.12 g (86.5%) of yellow crystals of X, mp 129-130°C. The compound is sparingly soluble in EtOH, MeOH, and insoluble in water and $CHCl_3$. IR spectrum, ν_{max}, cm^{-1}: 3400 (NH), 1650 (C=N) 1600 (CHar), 750, 690 (monosubstituted aromatic ring). Found: M^+ 197. $C_{12}H_{11}N_3$. Calculated: M 197.

(4-Pyridyl)glyoxylic Acid Thiosemicarbazone (XI). A solution of 0.33 g (3.7 mmoles) of thiosemicarbazide in 10 ml of water is added to a solution of 0.56 g (3.7 mmoles) of I in 10 ml of water, and the mixture is allowed to stand for 20 h at 20°C. The light-yellow precipitate is filtered, washed with water and EtOH. Yield, 0.82 g (98.8%) of XI, which is dissolved in 36.6 ml of 1 N NaOH, and the solution obtained is acidified with 36.6 ml of 1 N HCl. The precipitate obtained is filtered and washed with water. The yield of XI is 0.74 g (89.9%). Light-yellow crystals, mp 247-249°C. The compound is practically insoluble in water and in organic solvents. IR spectrum, ν_{max}, cm^{-1}: 1580, 1618, 1660 (C=N, C=O), 3060, 3092, 3190, 3285, 3375 (OH; NH). Found: M^+ 224. $C_8H_8N_4O_2S$. Calculated: M 224.

(4-Pyridyl)glyoxylic Acid Semicarbazone (XII). A solution of 0.41 g (3.7 mmoles) of semicarbazide hydrochloride and 0.31 g (3.7 mmoles) of sodium carbonate in 4 ml of water is added to a solution of 0.56 g (3.7 mmoles) of I in 4 ml of water. The reaction mixture is allowed to stand for 4 h at 20°C. The precipitate of XII is filtered, washed with water and EtOH. Yield, 0.7 g (84.3%) mp 218-219°C. White substance, which is sparingly soluble in water, and insoluble in organic solvents. IR spectrum, ν_{max}, cm^{-1}: 3460, 3340 (NH_2), 1700 (COOH), 1650 (NHCO) (Amide I), 1540 (amide II), 1300 (amide III), 1590 (CHar). Found: M^+ 208. $C_8H_8N_4O_3 \cdot H_2O$. Calculated: M 208.

(4-Pyridyl)glyoxylic Acid Phenylhydrazone (XIII). A 0.4 g portion (3.7 mmoles) of phenylhydrazine is added to a solution of 0.56 g (3.7 mmoles) of I in 2 ml of 35% ethanol. After 2 h at 20°C, the precipitate of XIII is filtered and washed with water. The yield of XIII is 0.75 g (84.3%). Yellow crystals, mp 165°C (dec). The compound is sparingly soluble in alcohols, and insoluble in water and $CHCl_3$. IR spectrum, ν_{max}, cm^{-1}: 1553, 1600 (C=N, C=O), 3290, 3265 (NH, OH), 750, 690 (monosubstituted Ar). Found: M^+ 241. $C_{13}H_{11}N_3O_2$. Calculated: M 241.

(4-Pyridyl)glyoxylic Acid 4-Phenylthiosemicarbazone (XIV). An aqueous-alkaline solution of the oxidation product of 5 g of 4-ethylpyridine is acidified to pH 3.0-4.0, the white precipitate is removed, and the filtrate is evaporated at 20°C, and extracted with EtOH. The ethanol extract containing 0.56 g (3.7 mmoles) of I, is added to 0.61 g (3.7 mmoles) of 4-phenylsemicarbazide in 15 ml of EtOH. The mixture is allowed to stand at 20°C for 20 h, the precipitate is filtered, and washed with water and ethanol. The yield of XIV is 0.94 g (85%). Yellow crystals, mp 170-171°C. IR spectrum ν_{max}, cm^{-1}: 3100 (NH), 2800-2600 (OH), 1720 (COOH), 1610 (CHar), 1560 (NHCS), 690-750 (monosubstituted Ar). Found: M^+ 300. $C_{14}H_{12}N_4O_2S$. Calculated: M 300.

3,5-Dioxo-(6-pyridyl)-2,3,4,5-tetrahydro-1,2,4-triazine (XVIII). A 0.7 g portion (3.1 mmoles) of XII is mixed with a solution of 1.36 g of potassium carbonate in 10 ml of water and the mixture is boiled for 9 h. The solution is filtered and acidified to pH 6.0-7.0 with 1 N HCl. The precipitate that separates is filtered, washed with water and EtOH. The yield of XVIII is 0.3 g (47%). Colorless crystals, mp 346-348°C (dec., from DMFA). The compound is soluble in DMFA, sparingly soluble in acetone, and insoluble in water, EtOH, MeOH, $CHCl_3$. IR spectrum, ν_{max}, cm^{-1}: 3220, 3120 (NH), 1700 (CO), 1600 (CHar). Found: M^+ 190. $C_8H_6N_4O_2$. Calculated: M 190.

5-Oxo-6-(4-pyridyl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine (XIX). A 0.5 g portion (2.2 mmoles) of XI is mixed with a solution of 1.1 g of potassium carbonate in 8 ml of water, and the solution obtained is boiled for 6 h. The reaction mixture is diluted with 2 ml of water, decolorized by carbon, and treated with 1 N HCl to pH 6.0-7.0. The precipitate that separates is filtered, washed with water and ethanol. The yield of XIX is 0.31 g (68.8%). Yellow crystals, mp 316-318°C. IR spectrum, ν_{max}, cm^{-1}: 3150, 3080, (NH), 1695 (CO), 1610 (CHar). Found: M^+ 206. $C_8H_6N_5OS$. Calculated: M 206.

2-Oxo-3-(4-pyridyl)-1,2-dihydroquinoxaline (XXII). A solution of 0.4 g (3.7 mmoles) of o-phenylenediamine is added to a solution of 0.56 g (3.7 mmoles of I in 3 ml of 95% alcohol. After stirring a light rose color appears, which rapidly passes into an abundant cream-colored precipitate. The temperature of the reaction mixture thus rises to 40°C. The mixture is allowed to stand for 20 h at 20°C. The precipitate is filtered and washed with water. The yield of XXII is 0.59 (72%). Light-yellow crystals, mp 199-201°C (from 50% EtOH). IR spectrum, ν_{max}, cm^{-1}: 1660 (NCO), 1610, 1600, 1590 (CHar). Found: M^+ 223. $C_{13}H_9N_3O$. Calculated: M 223.

EXPERIMENTAL (BIOLOGICAL)

The minimal inhibiting concentration (MIC) of the compounds studied was determined in a series of serial dilutions on a Soton medium, in comparison with the known antitubular preparation prothionamide. The tuberculosis mycobacteria was used as the test culture (strains $H_{37}R_V$, Academia, Bovinus 8, M. aquae SN632 and ATCC-607). The time of cultivation at 37°C was 14, 10, 10, 8, and 5 days, respectively. The results of the investigations are given in Table 2.

From Table 2 it is seen that compound XIII, for which the tolerance was determined in experiments on mice at a single daily intraperitoneal administration for 5 days, has the highest antitubercular activity. The maximal tolerable dose was 62.5 mg/kg. Further experiments on mice with experimental tuberculosis showed that the compound has no therapeutic antitubercular activity.

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